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Application of Electrophysiological Method to Study Interactions between Ibogaine and Cocaine

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ABSTRACT: The psychoactive indole alkaloid, ibogaine (IBO), has been investigated for over a decade concerning its reported anti-addictive properties for opioids as well as psychomotor stimulants. The mechanism for the anti-addictive action of IBO is still unclear. IBO interactions with opioid, NMDA, nicotinic, adrenergic, and serotonergic receptor sites have been suggested. The involvement of the dopaminergic system in IBO action is well documented. Increased or decreased levels of dopamine (DA) in specific brain regions following IBO pretreatment have been seen concomitantly with increased or decreased motor activity after subsequent amphetamine or cocaine administration. In this report, *in vivo* electrophysiological measures were monitored in awake adult male rats in order to investigate alterations of the electrocorticogram (ECoG) resulting from interactions between IBO and cocaine (COC). Rats were implanted bilaterally with bipolar ECoG electrodes. They were either injected with saline, COC alone (20 mg/kg, i.p.) or IBO (50 mg/kg, i.p.) and COC 1 hr later. The concentrations of DA, 5-HT, and their metabolites DOPAC, HVA, and 5-HIAA were assessed in the caudate nucleus in separate groups of saline-, COC-, and IBO/COC-treated rats. An α_1 power increase was observed within 10 min after COC injection, which lasted for less than 20 min. A desynchronization over α_2 and both beta power bands was observed throughout the recording. In IBO/COC-treated rats, a significant increase in delta, theta, and α_1 power occurred within 20 min after COC injection ($p < 0.05$). This effect lasted for up to an hour. DA levels significantly increased after COC only and decreased after IBO administration. A further decrease in levels of DA was observed in IBO/COC-treated rats. DA turnover increased significantly after IBO alone but was not observed after IBO/COC treatment. The alterations in ECoG and neurotransmitter levels suggest a decreased response to COC following IBO pretreatment.

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INTRODUCTION

Ibogaine (IBO), a psychoactive indole alkaloid, was originally derived from roots of the tropical rain forest shrub, *Tabernanthe iboga*. *T. iboga* along with related *T. manii* are widely used in the Central West African Gabon for religious (Bwiti, Mbiri) and medical purposes.¹ The chemistry of IBO and some of its actions have been known for almost a century. However, original observations made by Lotsof, and supported by a series of use patents issued in the United States on his methods for treatment of drug addiction, have recently intensified research into the anti-addictive properties of IBO and its metabolites.^{2–6}

The mechanism for the claimed anti-addictive actions of IBO is still unclear. Broad interactions between IBO and dopamine, serotonin, opioid, glutamate, nicotine, adrenergic, cholinergic, and other neurotransmitter receptor sites have been suggested.⁵ The involvement of the dopaminergic system is well documented. Increased or decreased levels of dopamine (DA) in specific brain regions following IBO pretreatment have been seen concomitantly with increased or decreased motor activity after amphetamine (AMPH) or cocaine (COC) administration.^{7–9} More recently, an intermediary role of neurotensin and the dopaminergic system in the anti-addictive actions of IBO against cocaine has been suggested.¹⁰

Electrophysiological methods may be helpful in studying mechanisms of IBO action. Analyses of the spectral patterns obtained from the recorded brain field potentials provide a sensitive tool for time-course studies of responses to different compounds acting on particular neurotransmitter systems. Dopaminergic neurotransmission was shown to be linked to the specific alterations in EEG profiles induced by cocaine.^{11,12} The present study was undertaken to analyze electrocorticogram changes resulting from interactions between IBO and COC, and to examine the response of the dopaminergic and serotonergic systems to the administration of COC following IBO pretreatment.

MATERIALS AND METHODS

Animals

Three-month-old male Sprague-Dawley rats of the Charles River cesarean delivered (CD) strain, obtained from the NCTR in-house breeding colony were used in this study. Animals were housed under controlled environmental conditions (temperature 22°C, relative humidity 45–55%, 12 hr light:dark cycle with lights off at 1800 hr) with *ad libitum* access to standard food and tap water.

Animal Instrumentation

Animals were anesthetized with isoflurane using a Stoelting (Wood Dale, IL) anesthesia system. Upon fixing the animal's head in a stereotaxic apparatus, the skin and muscle tissue on the upper cranium was incised to expose the skull. Four stainless steel screws were installed in 1.3 mm holes drilled in the cranial bone above the somatosensory cortex 3 mm laterally from the sagittal fissure and 1 and 4 mm posterior to bregma to serve as two bipolar electrocorticogram (ECoG) electrodes. The

electrodes were soldered to pins on a plug mounted onto the cranium. A ground wire soldered to one of the pins was placed in the dorsal neck.

Experimental Protocol

The ECoG was recorded via a tether and swivel system at least one week after cranial plug implantation. During recording, animals remained in a microdialysis bowl placed inside a Faraday cage. The signals were amplified using BioAmp 100 differential amplifiers and CyberAmp 380 signal conditioners (Axon Instruments, Inc.) to pass frequencies of 1–40 Hz and processed with LabView software (National Instruments, Austin, TX). The power spectra ($\mu\text{V}^2/\text{Hz}$) obtained by use of Fast Fourier transformations were divided into 1.25–4.50 Hz (delta), 4.75–6.75 Hz (theta), 7.00–9.50 Hz (α_1), 9.75–12.50 Hz (α_2), 12.75–18.50 Hz (β_1), and 18.75–35.00 Hz (β_2) frequency bands. After a 30-min morning baseline ECoG was recorded, rats were either injected with COC alone (20 mg/kg, i.p.) or pretreated with IBO (50 mg/kg, i.p.) followed an hour later by COC (20 mg/kg i.p.). The effects of treatments on dopamine (DA), serotonin (5-HT), and their metabolites were assessed in a separate group of rats sacrificed immediately after the corresponding time of recording (1 hr) following each treatment. The concentrations of DA, 5-HT, and their metabolites DOPAC, HVA, and 5-HIAA were examined in the caudate nucleus (CN) by HPLC-EC as previously described.¹³

Statistical Analysis

For individual animals, the median EEG power obtained during the baseline recording period was calculated for each frequency band. Similarly, median EEG power for each band was computed for 10-min time intervals following the baseline period (7 min immediately after injection). Relative power for each animal and band during the postbaseline period was calculated by dividing its median by the baseline median for an individual animal and band. A log-transform was then applied to these data to improve the distribution characteristics for the statistical analysis. For each 10-min time interval and each frequency band, the logarithm of the relative power was averaged between animals, and the resulting mean was compared to 1.0 (100%) using Student's *t* test. The resulting *p* value gives the statistical significance of any change in power within that frequency band as a result of the treatment. The level of statistical significance for this study was set at $p < 0.05$.

A one-way analysis of variance with Bonferroni's method of multiple comparisons was applied to test differences in monoamine levels between control and treated rats. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Administration of COC was accompanied by increased stereotypy (hyperactive sniffing, chewing) and locomotor stimulation within 10–15 min after the injection that lasted throughout the EEG recording. On the other hand, treatment with IBO alone inhibited locomotion and produced tremors. COC administration following IBO pretreatment appeared to increase locomotor activity but less than that observed

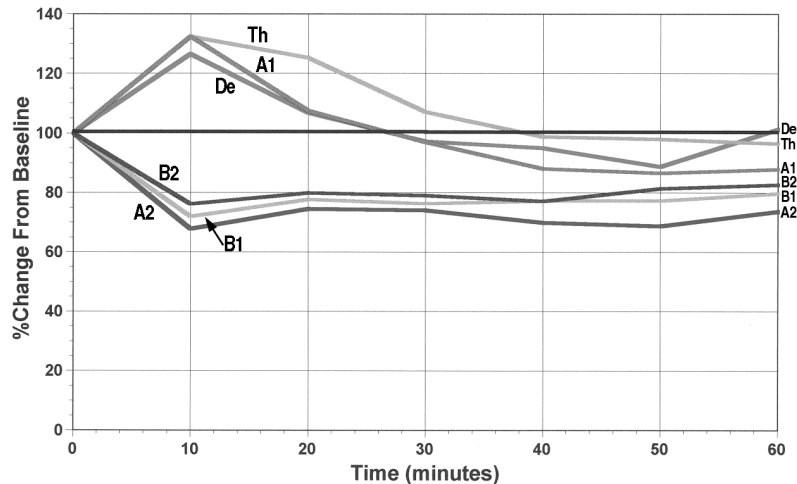


FIGURE 1. Delineation of time-course of effects produced by cocaine administration (20 mg/kg, i.p.) on cortical EEG power spectra. Relative power values calculated as percent of the 30-min baseline power assumed 100% in each band. De, delta (1.25–4.50), Th, theta (4.75–6.75), A1, α_1 (7.00–9.50), A2, α_2 (9.75–12.5), B1, β_1 (12.75–18.50), B2, β_2 (18.75–35.00).

after COC. Maisonneuve and colleagues⁹ reported inhibition of COC-induced hyperactivity when IBO was injected 1 hr prior to COC and potentiation when IBO was administered 19 hr before COC injection. Monitoring of the electrocorticogram (ECoG) in rats injected with COC only revealed a desynchronization over α_2 and both beta power bands throughout the 60 min of recording. An α_1 power increase within 10 min postcocaine injection was observed which lasted for less than 20 min (FIG. 1). Similar effects after COC injection (30 mg/kg, i.p.) were reported by Ali *et al.*¹² In rats treated with IBO and subsequently injected with COC, a significant increase in delta, theta, and α_1 power occurred within 20 min post COC injection (FIG. 2). This effect lasted for up to an hour.

Compared to saline-injected rats, COC injection significantly increased the DA level in the caudate nucleus. IBO treatment was associated with an increase in DA turnover. In IBO/COC-treated rats, a decrease in DA was observed, and the IBO effect on the DA turnover was abolished (TABLE 1). A trend toward an increase in 5-HT concentration was observed after COC administration. In rats treated with IBO, COC injection was associated with a decrease in 5-HT levels significantly below control values (TABLE 2).

The alteration of ECoG patterns and behavioral changes observed after COC administration were reported to result from activation of DA receptors in the striatum and prefrontal cortex by increased endogenous DA due to inhibition of DA reuptake.¹² DA neurons are modulated by multiple excitatory (GABA) and inhibitory (5-HT) neurotransmitters. The enhancement of the α_1 band after administration of large doses of COC (30 mg/kg) was suggested to result from effects of COC on adrenergic and serotonergic neurotransmission as well as additional activation of D_2

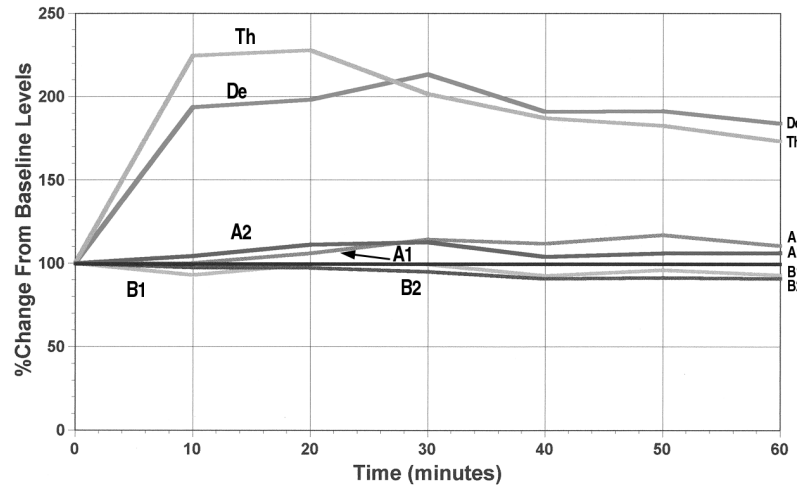


FIGURE 2. Delineation of time-course of effects produced by cocaine administration (20 mg/kg, i.p.) following ibogaine pretreatment (50 mg/kg, i.p.) on cortical EEG power spectra. Relative power values calculated as percent of the 30-min baseline power assumed 100% in each band. De, delta (1.25–4.50), Th, theta (4.75–6.75), A1, α_1 (7.00–9.50), A2, α_2 (9.75–12.5), B1, β_1 (12.75–18.50), B2, β_2 (18.75–35.00).

TABLE 1. Concentrations of dopamine (DA) and its metabolites DOPAC and HVA (ng/100 mg wet weight of tissue) in the caudate nucleus of rats treated with cocaine (COC) with or without ibogaine (IBO) pretreatment, (mean $\pm$ SEM)

Treatment	DA	DOPAC	HVA	DA turnover
Saline	822.5 $\pm$ 26.70	79.3 $\pm$ 4.28	60.2 $\pm$ 7.34	0.17 $\pm$ 0.02
COC	1008.8 $\pm$ 57.72*	88.0 $\pm$ 8.44	70.5 $\pm$ 9.47	0.16 $\pm$ 0.01
IBO	600.3 $\pm$ 48.89*	157.9 $\pm$ 25.82*	139.8 $\pm$ 21.90*	0.49 $\pm$ 0.06*
IBO+COC	277.7 $\pm$ 4.70*	28.6 $\pm$ 7.22*	29.5 $\pm$ 1.24*	0.21 $\pm$ 0.02

* $p < 0.05$.

receptors.^{12,13} The increase in α_1 power following hallucinogenic amphetamine derivatives has been attributed to a serotonergic effect via 5-HT receptors of cortical and striatal origin.¹⁴ In addition to this functional integration of cortex and striatum via the thalamus, other parts of the brain may also contribute to the ECoG alterations.

DA was found to decrease and 5-HT to increase in the striatum, prefrontal cortex, and nucleus accumbens within 1 hr after IBO administration.^{15,16} In a study where COC was administered 2 hr after IBO pretreatment, IBO significantly reduced DA and 5-HT release in nucleus accumbens, suggesting a role for the serotonergic system in modulating cocaine effects on dopaminergic neurotransmission.⁸ In the present study, COC injection 1 hr after IBO resulted in a decrease of DA and 5-HT

TABLE 2. Concentrations of serotonin (5-HT) and its metabolite 5-HIAA (ng/100 mg wet weight of tissue) in the caudate nucleus of rats treated with cocaine (COC) with or without ibogaine (IBO) pretreatment, (mean $\pm$ SEM)

Treatment	5-HT	5-HIAA
Saline	53.9 $\pm$ 3.99	19.53 $\pm$ 1.49
COC	61.3 $\pm$ 4.45	16.43 $\pm$ 1.75
IBO	43.2 $\pm$ 1.10	15.94 $\pm$ 1.78
IBO+COC	22.9 $\pm$ 1.14*	7.73 $\pm$ 0.93*

* $p < 0.05$.

in the CN. IBO was observed previously to decrease striatal DA within an hour after injection mainly by increasing DA metabolism and to decrease 5-HT and 5-HIAA.¹³ A similar pattern was observed in the present study. However, treatment with IBO/COC prevented the enhancement in DA metabolism observed after IBO (TABLE 1).

The ECoG alterations, mainly increased power in low frequency bands and enhancement of α_1 (7.00–9.50 Hz), indicate a contribution of the serotonergic system in IBO mediated response to COC. Recently, it has been reported that ibogaine may affect neurotensin systems.¹⁰ Neuropeptide, neurotensin, may antagonize cocaine actions via DA receptors, D₁ and D₂. As shown by actions of the kappa agonist (U50,488) and NMDA antagonist (MK-801) that imitate some effects of interaction between IBO and COC, other neurotransmitter receptor sites may also be involved in mediation of IBO interactions with COC as well.¹⁷ These systems may affect the overall biosignal response displayed in the ECoG.

CONCLUSIONS

Electrophysiological methods may be helpful in studying mechanisms of IBO action. The analysis of power spectra and neurotransmitter levels obtained after COC administration in rats pretreated with either saline or IBO 1 hr prior to COC administration suggest a contribution of the serotonergic system in IBO-mediated inhibition of the response to cocaine. However, other neurotransmitter systems may also be involved in the IBO/COC interaction in this experimental setting.

ACKNOWLEDGMENTS

This research was supported in part by an appointment (N.S.) to the Research Participation Program at the National Center for Toxicological Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

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